Amendments to PROTEIN STANDARD FOR ESTIMATING SIZE AND MASS
Application/Control Number: 10/068,663
Art Unit: 1734

a.) Introductory Comments

1. In ABSTRACT, the title "Protein standard for estimating size and mass" is deleted.
2. In ABSTRACT, the term "comprising" is changed to "containing" according to suggestion.
3. Change all "mass" to "amount" and all "masses" to "amounts" throughout the claims according to suggestion.

In part (a) of claim 1, the term "mass" is changed to "amount" according to suggestion.

In part (a) of claim 1, the term "known" is inserted before the term "size" and the term "amount" to make the claim specific.

In part (b) of claim1, the phrase "a range of at least separable by a given polyacrylamide gel electrophoresis" is changed to "a range that is separable by a given polyacrylamide gel electrophoresis" according to suggestion.

In part (c) of claim1, the phrase "a range of at least detectable by a given detection assay" is changed to "a range that is detectable by a given detection assay" according to suggestion.

In claim5, the phrase "the detecting intensity" is changed to "detection intensity" to address antecedent basis.

On line 2 of claim 5, the phrase "the polypeptide mass" is changed to "the polypeptide amount" according to suggestion.

Similarly, the term "mass" is changed to "amount" throughout the claims.

On line 2 of claim 6, the phrase "where the first container means" is changed to "where first container means" to address antecedent basis.

The phrase "to obtain relative positions and relative detection intensities of the polypeptides." is inserted at the end of part (b) in claim 7 according to suggestion—so

that the phrase "the relative positions" in part (c) of claim 7 and the phrase "the relative detection intensities" in part (d) of claim 7 have proper antecedent bases.

In part (a) of claim 10, the "a few polypeptides" is changed to "at least three polypeptides" to avoid indefiniteness according to suggestion.

The term "mass" in part (b) of claim 10 is changed to "amount".

In part (c) of claim 10, the phrase "with different sizes and masses" is changed to "that each has different size from one another and different amount from one another" according suggestion.

In claim 11, the phrase "protein standard is produced such that the standard contains at least three polypeptides" is changed to "detection assay is same as the detection assay according to claim 7" to avoid redundancy and to clearly describe the method.

In claim 15, the phrase "the range of their sizes" is changed to "the sizes of the polypeptides in the protein standard" to avoid indefiniteness.

In claim 16, the phrase "the range of their masses" is changed to "the amounts of the polypeptides in the protein standard" to avoid indefiniteness.

In claim 17, the phrase "the mass of each of the polypeptides is estimated by a protein assay" is changed to "the amount of each of the polypeptides is estimated by relative detection intensity of a protein assay"—so in claim 18 and claim 20, the phrase "the detection intensity" has proper antecedent basis.

The preamble of claim 18 is changed to "The method according to claim 17" according to suggestion.

In claim18, the phrase "the detection intensity" is changed to "detection intensity" to address antecedent basis.

In claim 19, the term "mass" is changed to "amount" according to suggestion.

In claim 20, the phrase "the detection intensity" is changed to "detection intensity" to address antecedent basis.

4. The present invention is not obvious over Houghton et al in view of ether the Boehringer Mannheim catalog (pages 219-221) or the Amersham Life Sciences catalog (pages 344-345).

Both Boehringer Mannheim catalog (pages 219-221) and the Amersham Life Sciences catalog (pages 344-345) teach molecular weight standards for protein that are only used for estimating size of the sample protein. None can also be used for estimating amounts of protein. There is no teaching of the claimed invention in these catalogs. In Fisher's Biotechnology Catalog, it states that "Amounts are balanced to ensure even staining intensity" which teaches away from disclosed invention (the disclosed invention needs different staining intensities to represent different amounts of proteins).

Houghton et al teach of a method for determining protein concentration in a protein sample by first separating the polypeptides with polyacrylamide gel electrophoresis (PAGE), followed by staining the separated polypeptides with a silver stain. The purified polypeptide is quantified by approximation of the staining intensity of the protein band on the gel to a series of known amounts (10-100 ng/protein band) of silver-stained molecular weight marker proteins. Houghton et al fail to teach that the molecular weight marker proteins also have different size from one another in addition to the different amount. This method is an assay to determine the amount of protein in a protein sample. Usually use bovine serum albumin (BSA), lysozyme, IgG or other proteins as molecular weight standard proteins to estimate the amount of sample protein. A series of known amount of protein such as 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ng are loaded in different lanes on the same gel with the sample protein in a separate lane. They are separated by PAGE and stained by a protein gel staining assay. The amount of sample protein is estimated by approximation of the staining intensity of the protein band on the gel. This method is only used to estimate the amount of the protein. They cannot be used to estimate the size (molecular weight) of the protein. The fact that Houghton et al fail to teach that they can estimate the molecular weight of the sample protein is because the assay can only estimate the amount of the protein. The fact that Houghton et al use the laborious method to determine the quantity of their sample protein indicates the disclosed invention is not obvious.

Now the question is whether it is obvious to combine regular commercial protein marker with Houghton et al teaching (or other similar teaching) to make the

quantitative protein standard in the patent application for those skilled in the art at the time the invention was made.

The applicant believes the claimed invention is not obvious for those skilled in the art at the time the invention was made. The reasons are following:

1). The disclosed quantitative protein standard is not simple combination of regular molecular weight (size) protein standard and method taught by Houghton et al. The simple combination of them is to run the regular size protein standard with series of known amount of molecular weight proteins such as 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ng on one gel. This will require 11 lanes on the gel (10 lanes for different amount of molecular weight proteins and 1 lane of regular size protein standard). If the gel has 12 lanes, only one protein sample may be loaded. This simple combination is laborious and costly. However it is commonly used when both size and amount of a sample protein need to be determined. With the claimed quantitative protein standard, only 2 lanes of the gel are needed to achieve the same result. Most lanes (10) of the gel may be devoted to other experiments. This significantly saves the labor and cost. The facts that the claimed invention is not a simple combination of previous arts and that those killed in the art use only the more laborious and costly method to determine their protein amount indicate the application is not obvious.

2). The method used only for estimating the amount of sample protein as taught by Houghton et al has been available since the publication of Fishbein (Anal. Biochem., 46, 388-401, 1972) which is over 30 years (Baltimore et al used similar assay 10 years ago). Coomssie Blue was used for protein staining after electrophoresis about 40 years ago (Groth et al, Biochim. Biophys. Acta 71, 377, 1963). Because of obvious advantages of saving labor and cost with the claimed invention, those skilled in the art surely would have implemented it by now. The fact of lack of implementation for over 30 years indicates the claimed invention is not obvious.

3). The experimental observations teach away from the disclosed application. It is observed that same amount of different proteins give different staining intensities by a staining assay. The staining intensities can be 3 to 5 times different. It is also observed different proteins with same staining intensity can contain different amount of protein. The amount of protein can be 5 to 10 times different. Because of these

observations, people would not think it is possible to make the invention. For example, when 10, 5, and 3 ug of different proteins are mixed together. The ratio of their staining intensities by a given assay may not be 10:5:3. It may be 3:5:10 when the third protein stains strongest and the first protein stains weakest. It may also be 20:5:1 when the first protein stains strongest and the third protein stains weakest. These experimental observations teach away from the disclosed application. The applicant devised new principles of operation to solve these problems: (a) the absolute amount of each protein is not relevant in the quantitative protein standard. (b) the relative staining intensity of each protein will represent its quantity against a standard protein such as BSA or lysozyme. In other words, the relative amount of protein obtained from the relative staining intensity does not reflect the amount of each protein in the quantitative protein standard but represent the relative amount of protein of the standard protein such as BAS or lysozyme. (c) the staining assay used to prepare the quantitative protein standard should be similar to the assay using the protein standard to make the quantification reliable and consistent. This means that if the quantitative protein standard is made by Coomassie Blue staining, the standard should only be used by Coomassie Blue staining assay. Using it in silver staining assay will not be accurate in estimating the protein amount. The quantitative protein standard was made possible only after these new principles of operation were used. Involvement of new principles of operation indicates the disclosed invention is not obvious.

4). The invention solves a long-felt, long-existing, but unsolved need. The applicant has been working on protein sizing and quantification for many years. It always painful for the applicant to use the laborious method taught by Fishbein (similar as that taught by Houghton et al). This laborious and costly method is used today in many academic and industrial labs as revealed recently by Houghton et al. Therefore solution of the long-felt and unsolved need further indicates the application is not obvious.

In conclusion, the disclosed invention is not obvious over any previous arts or combination of them. Therefore Claims 1, 5-11 and 15-20 are patentable. Claims 2-4

and 12-14 are dependent to Claims 1, 5-11 and 15-20. They are provided for fully disclosure of the invention, therefore they are also patentable.

5. The teaching by Baltimore et al is similar as that by Houghton et al. It can only determine the amount of the protein. Baltimore et al does not teach the claimed invention.
6. Murray et al teach the method can only estimate the size (molecular weight) of the sample protein. Murray et al does not teach the claimed invention.
7. Baba et al teach a quality control material contain recombinant human serum albumin. Baba et al does not teach the claimed invention.